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Comparison with Rats

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16. ABSTRACT Mice were injected intratracheally with silica or Mt. St. Helens volcanic ash (0.2 mg/g body weight) and examined 6 months later for changes in lung function, histology, and hydroxyproline content. Results were compared with a similar study using rats. Mice injected with volcanic ash showed significant changes only in wet lung weights. Those injected with silica showed an approximate doubling of lung wet weight and dry weight and hydroxyproline content. Larger increases were seen if lymph nodes were left attached. Lung compliance, total lung capacity and the shape of the pressure-volume curve of the lung were changed as much as 22% in the silica treated mice. A mild degree of fibrosis with no dense lung consolidation was noted microscopically in silica treated mice. In contrast, silica treated rats showed dense lung consolidation, 3-5 fold increases in lung weights and hydroxyproline content, and up to 40% reductions in pulmonary function measurements. It is concluded that Swiss albino mice develop a milder degree of fibrosis than similarly treated Sprague-Dawley rats and that both biochemical and functional indicators are effective in detecting pneumoconiosis in these species.		
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FUNCTIONAL AND BIOCHEMICAL INDICATORS OF PNEUMOCONIOSIS IN MICE: COMPARISON WITH RATS

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Mice were injected intratracheally with silica or Mt. St. Helens volcanic ash (0.2 mg/g body weight) and examined 6 mo later for changes in pulmonary function, histology, and hydroxyproline content. Results were compared with a similar study using rats. Mice injected with volcanic ash showed significant changes only in wet lung weights. Those injected with silica showed an approximate doubling of lung wet weight and dry weight and hydroxyproline content. Larger increases in lung weight were seen if lymph nodes were left attached. Lung compliance, total lung capacity, and the shape of the pressure-volume curve of the lung were changed as much as 22% in the silica-treated mice. A mild degree of fibrosis with no dense lung consolidation was noted microscopically in silica-treated mice. In contrast, silica-treated rats showed dense lung consolidation, threefold to fivefold increases in both wet and dry lung weights and hydroxyproline content, and up to 40% reductions in pulmonary function measurements. It is concluded that Swiss albino mice develop a milder degree of fibrosis than similarly treated Sprague-Dawley rats and that both biochemical and functional indicators are effective in detecting pneumoconiosis in these species.

INTRODUCTION

Various animal species have been used for studying the fibrogenicity of inhalable particles. A large body of data is available using rats (Zaidi, 1969; Reiser et al., 1982). Guinea pigs injected with silica also developed pulmonary fibrosis comparable to that of humans (Dauber et al., 1980). Mice have been reported to develop typical fibrotic lesions following inhalation or intravenous injection of silica (Burns et al., 1980; Zaidi,

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1969; Gardner, 1938), bleomycin (Sikic et al., 1978), or treatment with butylated hydroxytoluene and 70% oxygen (Haschek and Witschi, 1979). However, some reports indicate that species differences exist in the extent of the pulmonary fibrosis induced by particles. Hamsters do not appear to develop typical fibrosis following injection of silica (Gross et al., 1967). Mice, guinea pigs, and rabbits treated with hydrated alumina either intratracheally or intravenously were reported to develop a low to moderate fibrogenic response, while rats so treated developed severe pulmonary fibrosis (Engelbrecht et al., 1959). A poor fibrogenic response occurred in mice intratracheally injected with silica, asbestos, kaolin, mica, and talc (Sahu et al., 1975a,b, 1978).

Mice offer the advantage of inexpensive housing, a consideration that becomes significant when irreversibility of the lung lesion is of interest and long waiting periods after injection are employed. Also, the desirability of performing dose-effect studies increases the number of animals required, making mice an attractive animal for fibrosis studies.

A recent project in our laboratory that examined the fibrogenic activity of Mt. St. Helens volcanic ash in rats (Raub et al., 1983) provided an opportunity to perform a similar study concurrently using mice, the results of which are presented here. Since previous studies of pneumoconiosis often have suffered from problems of quantification of the response, we applied pulmonary function and biochemical tests in addition to the routine histopathologic evaluation.

METHODS

Animals and Chemicals

Female Swiss albino mice (specific-pathogen-free, cesarean-derived) weighing 18–23 g (approximately 4–5 wk old) were obtained from Charles River Breeding Labs, Wilmington, Mass. Mice were housed on heat-treated white pine shavings in filter-top polycarbonate cages and fed Wayne lab diet *ad libitum*.

Silica (Minusil, $<5 \mu\text{m}$) was purchased from Pennsylvania Glass Sand Corp., Pittsburgh, Pa. Volcanic ash was collected by Dr. Andrew McFarland in Ritzville, Wash., from the first eruption of Mount St. Helens in June 1980. It was fractionated to obtain the sample used here, which had a mean particle size $<2.5 \mu\text{m}$ (Graham et al., 1983). Silica and volcanic ash suspensions were made 10% w/v in 0.9% saline for injection (Abbott Laboratories, North Chicago, Ill.).

Intratracheal Injection Procedure and Experimental Regimen

Mice were anesthetized with 5% halothane (Halocarbon Laboratories, Inc., Hackensack, N.J.) and given a single intratracheal injection of 50 μl

volume of saline, volcanic ash, or silica. The procedure used for injections has been described previously (Hatch et al., 1981). Lungs used for microscopy were fixed by introduction of 2% glutaraldehyde in 0.84 *M* sodium cacodylate buffer, pH 7.4, into the airways at 30 mm pressure (Matthau et al., 1978).

Pulmonary Function Testing

Six mice from each group were evaluated. The animals were anesthetized by an intraperitoneal injection of 50 mg sodium pentobarbital/kg. A tracheostomy was performed by tying a 17-gauge luer stub adapter (Becton-Dickinson, Rutherford, N.J.) in the airway. Mice were then placed in a whole-body plethysmograph for pulmonary function measurements. Lung volume was obtained from changes in plethysmograph pressure measured by a differential pressure transducer (Setra Systems, Model 234, Natick, Mass.). Airway pressure was measured by a differential pressure transducer (Validyne, Model MP-45, Northridge, Calif.) referenced to the plethysmograph providing the pressure difference across the total respiratory system (P_{rs}). Following a short equilibration period of resting breathing, the airway was occluded at end-expiration, and end-expiratory volume was calculated using Boyle's law. The animals were hyperventilated on a small-animal respirator (Ealing Corp., Model 5255, Natick, Mass.) to increase minute ventilation and establish a uniform volume history. A pressure-volume curve was generated by inflating and deflating the lungs with a mass flow controller. Vital capacity was measured between a P_{rs} of -20 cm H_2O and $+30$ cm H_2O . Respiratory system compliance was calculated from a nonlinear least squares regression fit of the deflation pressure-volume curve. The pressure obtained at the linear portion of this curve was designated the inflection pressure. Multiple-breath nitrogen washout curves were performed by ventilating the animals at a fixed volume and frequency with 100% oxygen while recording breath-to-breath nitrogen concentration (Med Science Nitralyzer, Model 505, St. Louis, Mo.). The slope of the log end tidal nitrogen concentration versus breath number was used to assess the distribution of ventilation. Single-breath diffusing capacity for carbon monoxide and residual volume were measured by gas dilution techniques previously described by this laboratory (Takezawa et al., 1980). Total lung capacity was obtained by the addition of vital capacity and residual volume. Specific compliance was obtained by expressing the respiratory system compliance as a percentage of total lung capacity. All data were processed by a digital computer (PDP 8, Digital Equipment Corporation, Maynard, Mass.).

Lung Collagen (Hydroxyproline) Assay

Mice anesthetized with halothane were exsanguinated through the abdominal aorta and the lungs. Lungs were weighed and then dried in a $110^{\circ}C$ oven. Dry weights of the lungs were determined and 10 ml of 6 *N*

HCl containing [^{14}C]hydroxyproline for recovery determination (New England Nuclear, Boston, Mass.) were added to the screw-cap tubes, and the tissues were hydrolyzed overnight at 110°C . Then 1 ml of 20% w/w Norit carbon suspension in water was added to each tube prior to filtration of the hydrolysates. The colorimetric assay for hydroxyproline in the hydrolysates followed the method of Kivirikko et al. (1967), except that the chloramine-T was dissolved in the borate buffer and *n*-propanol was substituted for 100% ethanol in the Erlich's reagent. These changes simplified the method without affecting the results obtained.

Statistical Analysis

Pulmonary function measurements were evaluated statistically by comparing experimental with control body-weight-adjusted means of each data point using a one-way analysis of covariance. Hydroxyproline and lung weight data were analyzed using one-way analysis of variance. When *F* values indicated significance ($p < 0.05$), comparisons of each group with the sham control were made using the test of Dunnett (1955). Determination of lowest effective concentration in the silica dose-effect study was done according to Williams (1972).

RESULTS

Gross and Histologic Observations

The lungs of mice evaluated 6 mo after injection had pale-colored nodules visible on the surfaces that were very obvious in silica-treated animals and barely visible in lungs treated with volcanic ash. The lungs of saline-injected mice were grossly normal. Enlarged tracheobronchial lymph nodes were most noticeable in silica-injected mice, but they were also seen in mice injected with volcanic ash. Microscopically, the lungs of silica-injected mice had a mild degree of fibrosis but little or no dense consolidation of pulmonary tissue. Multifocal areas of necrosis contained birefringent particles, cholesterol clefts, and mononuclear cell accumulations. Silica-treated mice also had increased numbers of alveolar macrophages and eosinophilic debris in the alveoli. Tracheobronchial lymph nodes were enlarged and contained multifocal areas of necrosis, birefringent particles, macrophages accumulation, and lymphocytic hyperplasia. Pulmonary lesions in mice given volcanic ash were much less severe than those in silica-treated animals. The lymph nodes did not have focal areas of necrosis, but were enlarged and had accumulations of mononuclear cells.

Pulmonary Function Measurements

The mice receiving 5 mg volcanic ash had no significant changes in pulmonary function when compared to the saline-injected controls (Table

TABLE 1. Lung Biochemistry and Function of Mice following Intratracheal (Injection of Volcanic Ash or Silica)^a

Parameter	Saline	Volcanic ash	Silica
Breathing frequency (per minute)	97 ± 7	85 ± 7	92 ± 7
Tidal volume (ml)	0.19 ± 0.0	0.21 ± 0.0	0.16 ± 0.0
Minute ventilation (ml/min)	17.9 ± 1.6	17.5 ± 1.5	14.7 ± 1.6
End expiratory volume (ml)	0.50 ± 0.04	0.51 ± 0.04	0.49 ± 0.04
Residual volume (ml)	0.39 ± 0.02	0.42 ± 0.01	0.34 ± 0.02
Vital capacity (ml)	1.18 ± 0.06	1.15 ± 0.06	1.00 ± 0.06
Total lung capacity (ml)	1.57 ± 0.06	1.57 ± 0.06	1.35 ± 0.06 ^c
Respiratory system compliance (cm H ₂ O)	0.073 ± 0.005	0.068 ± 0.004	0.058 ± 0.005 ^b
Specific lung compliance (%TLC/cm H ₂ O)	4.82 ± 0.22	4.22 ± 0.22	4.02 ± 0.26
Inflection pressure (cm H ₂ O)	4.54 ± 0.23	4.22 ± 0.22	3.16 ± 0.24 ^c
Multiple-breath nitrogen washout (log %N ₂ /breath)	0.316 ± 0.037	0.247 ± 0.036	0.323 ± 0.039
Single-breath diffusing capacity (ml/min·torr)	0.029 ± 0.002	0.030 ± 0.002	0.029 ± 0.003
Lung wet wt. (mg)	241 ± 20	396 ± 50 ^c	919 ± 31 ^c
Lung dry wt. (mg)	65 ± 6	92 ± 12	224 ± 27 ^c
Lung hydroxyproline (μg/lung)	709 ± 134	721 ± 50	1326 ± 50 ^c

^aData reported as mean ± standard error of the mean. Mice were injected with 5 mg (0.2 mg/g body weight) of ash or silica in 50 μl volume and examined 6 mo later.

^bSignificant difference ($p < 0.10$) from control value.

^cSignificant difference ($p < 0.05$) from control value.

1). Mice receiving silica had a smaller lung volume, and a reduced lung compliance and inflection pressure (Fig. 1). None of the other parameters measured were significantly affected by silica exposure.

Lung Hydroxyproline Levels

Lung hydroxyproline levels were determined in the same mice used for pulmonary function measurements (Table 1), and in a separate experiment in which the doses of instilled silica were varied (Table 2). Body weights prior to sacrifice were higher in the first experiment, although they were not significantly affected by silica or ash treatments. Mean body weights for experiments 1 and 2 were 32.0 ± 3.5 (SD) g and 37.2 ± 21 (SD) g respectively.

In the first experiment, hydroxyproline content in lungs of silica-treated mice 6 mo after injection was significantly increased to levels nearly twice those seen in the lungs of saline-injected mice. Lungs from mice injected with volcanic ash were not significantly different from controls (Table 1). Lung plus lymph node wet weights were increased 3.6-fold in silica-treated mice and 1.6-fold in volcanic ash-treated animals. Dry lung weights were increased 3.4-fold in silica-treated animals, while the 1.4-fold increase in the dry weight for the volcanic ash-treated mice was not statistically significant. When either hydroxyproline content or

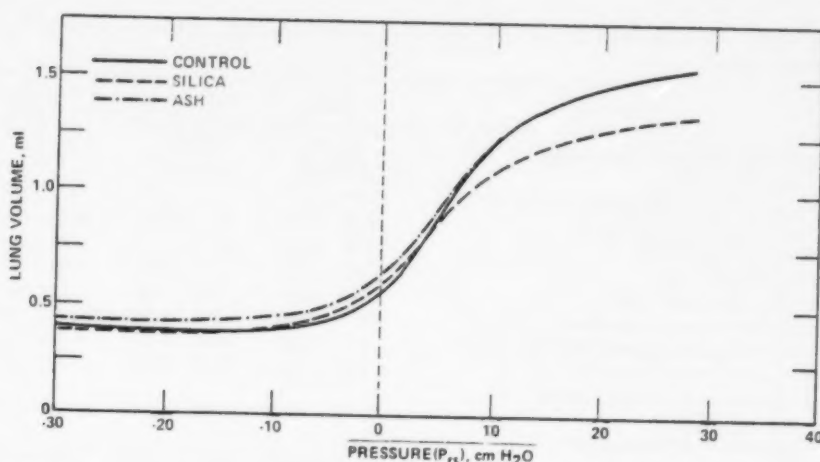


FIGURE 1. Pressure-volume curve of the respiratory tract averaged from mice injected with 50 μ l saline (control) or with 5 mg silica or volcanic ash in 50 μ l saline, 6 mo previously.

lung weights from individual animals were compared with any of the affected pulmonary function measurements on the same animals, no relationship was apparent.

In the second experiment, smaller increases in most measurements were found as compared to what was seen in the first experiment, probably because the lymph nodes were removed (Table 2). Smaller increments were seen in all measurements at 77 d compared to 181 d postinjection. Lung hydroxyproline content was increased by silica in a dose-dependent fashion. The lowest dose of silica that produced a significant increase in hydroxyproline content at 181 d was 0.5 mg/lung. Wet and dry lung weights were also increased by the silica injections; however, significant effects on these parameters did not occur until a dose of 2.5 mg/mouse was reached. Hydroxyproline concentrations expressed per gram of wet lung rather than per lung were not significantly altered by silica injections in this experiment, although the values were lower in the first study.

Comparison of Mice with Rats

Mice that received 5 mg silica or volcanic ash intratracheally were compared with rats that received a similar dose as a function of body weight (0.2 mg/g body weight). Data for rats, which are reported in detail elsewhere (Raub et al., 1983), showed severe consolidation of lung tissue and nodular fibrosis. Quantitative differences between the responses in these species are shown in Table 3. Body weights were reduced as a result of silica injection in rats but not in mice. Lung wet weights were increased by silica about twofold to fourfold in mice and fourfold to fivefold in

TABLE 2. Lung Weights and Hydroxyproline Levels in Mice following Intratracheal Injection of Various Amounts of Silica

Condition	n	Lung wt. (g)	Lung dry wt. (g)	Hydroxyproline levels (μ g/lung)
77 d postinjection				
Saline	3	0.156 $\pm$ 0.006	0.034 $\pm$ 0.000	260.7 $\pm$ 5.1
Silica 0.05 mg/mouse	3	0.159 $\pm$ 0.005	0.032 $\pm$ 0.001	255.9 $\pm$ 4.6
0.25 mg/mouse	3	0.179 $\pm$ 0.019	0.037 $\pm$ 0.005	273.3 $\pm$ 30.3
0.50 mg/mouse	3	0.175 $\pm$ 0.003	0.037 $\pm$ 0.001	280.5 $\pm$ 7.4
2.50 mg/mouse	3	0.225 $\pm$ 0.166 ^a	0.047 $\pm$ 0.004 ^a	352.4 $\pm$ 7.5 ^a
5.00 mg/mouse	3	0.215 $\pm$ 0.021	0.046 $\pm$ 0.006	356.5 $\pm$ 47.0
181 d postinjection				
Saline	7	0.171 $\pm$ 0.006	0.037 $\pm$ 0.002	418 $\pm$ 32
Silica 0.05 mg/mouse	7	0.179 $\pm$ 0.007	0.039 $\pm$ 0.002	421 $\pm$ 23
0.25 mg/mouse	7	0.194 $\pm$ 0.005	0.042 $\pm$ 0.001	438 $\pm$ 26
0.50 mg/mouse	7	0.194 $\pm$ 0.016	0.042 $\pm$ 0.004	494 $\pm$ 34 ^a
2.50 mg/mouse	7	0.223 $\pm$ 0.014 ^a	0.048 $\pm$ 0.003 ^a	586 $\pm$ 28
5.00 mg/mouse	7	0.316 $\pm$ 0.030	0.071 $\pm$ 0.006	629 $\pm$ 29

^aLowest effective concentration determined by Williams test. Mean $\pm$ SE are given.

TABLE 3. Comparison of Mice and Rats in Pulmonary Response to Silica or Volcanic Ash Injection

Parameter measured	Effects due to particle injection (treated/control $\times$ 100)			
	Silica	Ash	Silica	Ash
Body wt.	93.2	96.9	82.0 ^a	48.2
Lung wet wt.	244.5 ^a	164.3 ^a	414.9 ^a	104.7
Lung dry wt.	377.7 ^a	141.5	502.6 ^a	112.8
Hydroxyproline (per lung)	187.4 ^a	101.6	646.9 ^a	129.6
Breathing frequency	94.8	87.6	107.8	92.8
Tidal volume	84.3	110.5	75.3 ^a	103.3
End expiratory volume	98.0	105.2	103.2	109.1
Residual volume	87.2	107.7	93.3	101.0
Vital capacity	84.7	97.5	63.7 ^a	91.9
Total lung capacity	86.0 ^a	100.0	66.5 ^a	92.8
Respiratory system compliance	79.4 ^a	93.2	58.1 ^a	91.2
Specific lung compliance	83.4	89.8	54.2 ^a	101.6
Inflection pressure	69.6 ^a	92.9	77.6 ^a	98.1
Multiple-breath N ₂ washout	102.2	78.2	70.0 ^a	87.5
Single-breath diffusing capacity	100.0	103.4	90.0	119.5

^aSignificant differences at $p < 0.05$ from control by Dunnett's test on raw values (3-8 animals per group). Rats were exposed and examined similarly to the mice.

rats. An important difference between rats and mice was the extent of the increase in hydroxyproline content induced by silica. While mice showed a less than doubling of these levels in both experiments, rats showed a 5.5-fold increase. Increases in lung hydroxyproline were not seen following volcanic ash in either species.

Seven of the pulmonary function measurements were significantly decreased in rats, while only 3 were decreased in mice. Decreases in total lung capacity, lung compliance, and vital capacity seen in silicotic rats ranged from 22 to 46%, while mice showed decreases of only 14-25%.

DISCUSSION

The present study examines whether mice could be used for quantifying the fibrogenicity of inhalable particles. Mice developed only a mild degree of histologically detectable fibrosis, in agreement with previous studies (Sahu et al., 1975a,b, 1978). Lung wet weights were increased in mice following both silica and volcanic ash injection. The extent of removal of lymphoid tissue was critical in evaluation of lung weight changes. Injection of different amounts of silica into mice yielded a dose-related change in lung wet and dry weights and hydroxyproline levels. Increased hydroxyproline content, which is assumed to be indicative of increased lung collagen, was seen only in silica-injected mice. Since increased lung weight usually seems to accompany increased hydroxyproline content, the hydroxyproline concentrations (per gram of lung) did not increase, in agreement with previous studies on humans and animals (Bailey et al., 1964; McCullough et al., 1978; Crystal et al., 1976).

Pressure-volume relationships of the lung, as well as diffusing capacity, have been used clinically to follow the progression of pneumoconiosis (Gibson and Pride, 1977). The increase in interstitial tissue in this disease is measurable as decreased lung volumes, compliance, and diffusing capacity, and increased elastic recoil of the lung. The decreased total lung capacity and lung compliance and the changes in the pressure-volume curve seen here in silica-treated mice are consistent with the clinical observations. Failure to measure a change in other parameters such as diffusing capacity is probably a reflection of the scale differences in the thickness and size of airways between humans and mice, as well as technical difficulties of performing pulmonary function measurements on mice. Also, in contrast to studies with humans, maneuvers performed in mice were done by forced inflation and deflation in the anesthetized animal. The normal values for lung compliance and total lung capacity in mice compare favorably with the studies that have been published in the recent literature (Vinegar et al., 1979; Fisk and Kuhn, 1976; Szapiel et al., 1981).

Comparison of the mouse data with that obtained in rats indicate a large species difference. The density and extent of lung consolidation

observable by gross and microscopic observation of thin sections of the lungs was much greater in rats than in mice. The wet and dry lung weights were also increased to a greater extent in rats, although the hydroxylproline content was the most obvious example of the difference between the two species. Pulmonary function changes were also more prevalent and severe in rats than in mice. These findings suggest that although mice are much less expensively housed, the mild degree of fibrosis they develop would probably make them less sensitive for evaluation of particles that are only mildly fibrogenic. However, it is possible that other strains of mice would develop as severe a fibrosis as rats. The species differences observed might also be due to differences in the incidence of mild pulmonary infection during the 6 mo following injection of the particles (Holt, 1974; Zaidi, 1969). It is difficult to rule out the possible contribution of these infections to the development of fibrosis, even though histopathology appeared normal. Differences in the time course of development of the fibrotic lesion could also exist: rats may develop the lesion faster than mice.

Species differences in the development of fibrosis may provide clues as to the mechanism of development of this effect. In previous papers (Hatch et al., 1980a,b) we have shown that silica and asbestos stimulate oxidant production in alveolar macrophages, and that macrophages from mice exhibit a smaller response than rats when stimulated with asbestos. Evidence is accumulating that oxidant release in the lung may have a contributory role in the development of fibrosis (Bhatnagar, 1977; Johnson et al., 1981).

In summary, we report that commonly used albino laboratory mice and rats have a different pulmonary fibrogenic response to silica, with mice having the weaker response. Pulmonary function and biochemical indicators of fibrosis appear to effectively quantify the fibrosis in both mice and rats. Of the quantitative methods used, the hydroxypyrrolone content of the lung appeared most affected; it was specific for the effects of silica, and was relatively easy to perform.

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